PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
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PERIOD COVERED: November, 1989

I. GLUTATHIONE DEPLETION ASSAY

A. Objective: To evaluate the role of arachidonic acid metabolism in the reduction of glutathione by 2R1 CSC in V79 cells.

B. Results: Treatment with indomethacin (1 to 100 μM), an inhibitor of cyclooxygenase activity, showed variable effects on the reduction of glutathione by 2R1 CSC. Two previous experiments showed indomethacin to prevent glutathione reduction by CSC by approximately 40% and 70%. Most recent results showed indomethacin to increase glutathione reduction by CSC by up to two-fold. Treatment with nordihydroguaiaretic acid (NDGA; 0.1 to 10 μM), an inhibitor of lipoxygenase activity, prevented glutathione reduction by CSC by approximately 35%. A previous experiment had shown NDGA to prevent glutathione reduction by CSC by approximately 50%. No dose response was seen for indomethacin or NDGA. Indomethacin or NDGA alone did not alter cellular glutathione levels.

C. Plans: The testing of other inhibitors is under consideration.

D. Reference:

McCoy, W. R. Notebook No. 8739, pp. 185-187.

II. JB6 MOUSE EPIDERMAL CELL TRANSFORMATION ASSAY

A. Objective: To implement the JB6 mouse epidermal cell transformation assay in our laboratory.

B. Results: A third soft agar assay testing TPA was set up, and all three TPA tests were counted for colony formation. Two plate growth toxicity experiments using mezerein and two using phorbol diacetate were completed. Results correlated with dose levels reported in the literature for soft agar assays. One benzoyl peroxide soft agar experiment was completed. There appeared to be some problems with that particular test, but it was not possible to determine the precise source(s) of the difficulty from the experimental data. Two mezerein soft agar assays were begun.

C. Plans: Statistical analysis of the TPA results will be completed by J. Tindall. A second benzoyl peroxide soft agar assay will be set up in a manner designed to determine the source(s) of problems in the original experiment. A third mezerein soft agar assay will be started. An automatic colony counter will be evaluated for possible use in facilitating this assay.

D. **Reference**:

Nixon, G. M. Notebook No. 8711, p. 149.

III. ACQUISITION AND MAINTENANCE OF CELL LINES

A. **Objective**: To acquire and maintain a variety of cell lines for use in biochemical assays.

B. **Results**: New frozen 3T3 cells from ATCC are doing well. Results from Microbiological Associates indicated that mycoplasma was found in our 3PC cell line, while no mycoplasma was found in all other cell lines tested. Another batch of the 3PC cell line and a sample of the MT 1/2 cell line were sent to ATCC for mycoplasma testing. The generation time of each JB6 cell clone has decreased. The JB6 cell clones are being monitored closely in conjunction with the transformation assay.

C. **Plans**: Continue to monitor each cell line.

D. **References**:

Burruss, T. J. Notebook No. 8896, p. 14.
Vaughan, B. G. Notebook No. 8828, p. 85.

IV. EGF BINDING ASSAY

A. **Objective**: To examine the role of phospholipid metabolism in the inhibition of EGF binding caused by CSC.

B. **Results**: A second experiment with ETYA, an inhibitor of both lipoxygenase and cyclooxygenase activities, was conducted this month. As with the first experiment, only a minimal effect of ETYA on EGF binding was observed following treatment with ETYA alone, and there were no significant effects of ETYA on the response of 2R1 CSC.

C. **Plans**: To examine the effects of PKC down regulation and the role it plays with CSC on EGF binding.

D. **Reference**:

Stagg, D. L. Notebook No. 8883, p. 66.